



Universidade de São Paulo
Instituto de Química



Análise de Microbiomas aula 2

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2024

Classificação de reads de DNA total

- Similaridade com sequências de origem conhecida
 - BLAST
- Propriedades intrínsecas de cada sequência
 - Assinaturas genômicas
 - Apropriado para binning

Por analogia com classificação de reads em dados de 16S (OTUs)

- Separar reads em “caixinhas”
- no caso de OTUs, cada caixinha tem os reads que mutuamente se parecem num nível de 97 ou 98% de identidade
- qual seria o análogo para DNA total?

Classificação com base na frequência de palavras de k bases

$k = 4$: AAAA, AAAC, AAAG, AAAT, CAAA, etc...

Dada uma janela de x kb, podemos contar as ocorrências de cada uma dessas palavras dentro da janela

Exemplo:

AG**ATT**A GCGACT**ATT**A TAGCCTAGATCGATC**ATT**A CC

AGAT ocorre 2 vezes

ATTA ocorre 3 vezes

etc

Palavras de k bases: k-mers (kâmeros)

Matriz de frequências

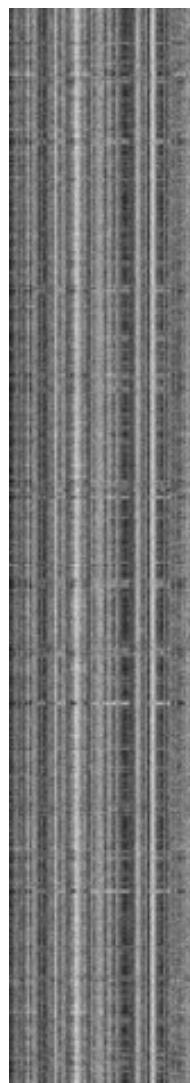
janela	AAAA	AAAC	AAAG	AAAT	ACAA	ACAC	ACAG	ACAT
1	15	2	9	...	0			
2	16	3						
3	14	0						
4	13	2						
5	15	4						
6	12	0						
7	18	1						
8	17	3						
9	16	1						

Exercício

- $S_1 = \text{TTCTACTACT}$
- $S_2 = \text{TTGTACTAGG}$
- $S_3 = \text{ACTTCTACTA}$
- Montar as matrizes de frequências para essas 3 sequências, supondo palavras de tamanho 2
- Quais duas sequências são mais similares entre si em termos das frequências dessas palavras?

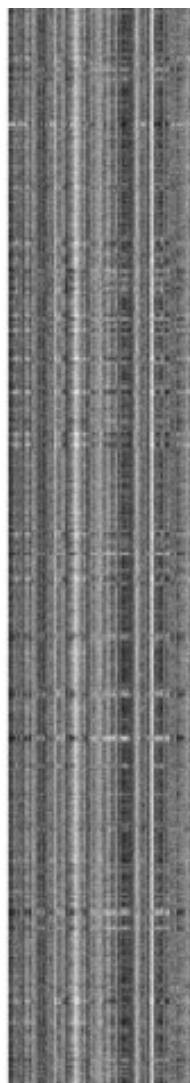
Genome “barcodes”

Burkholderia pseudomallei



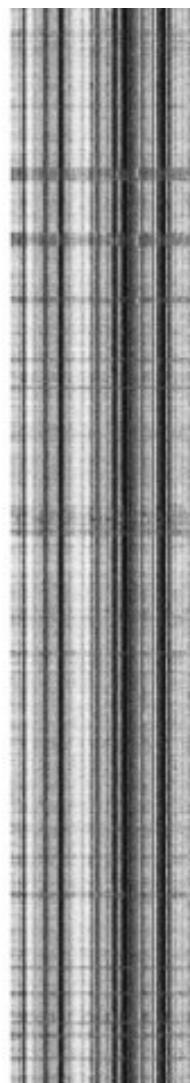
(a)

E. coli K12



(b)

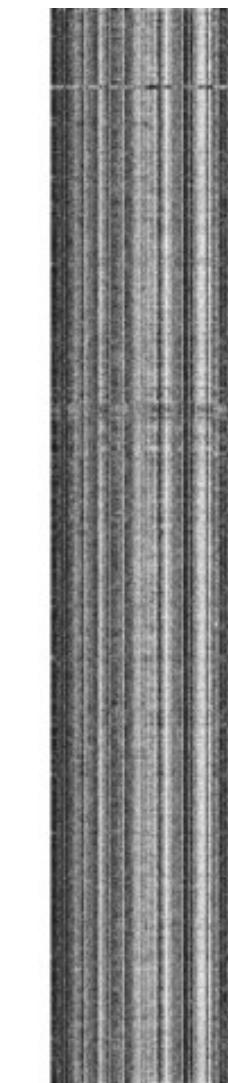
E. coli O157



(c)

Pyrococcus furiosus

(d)



(e)



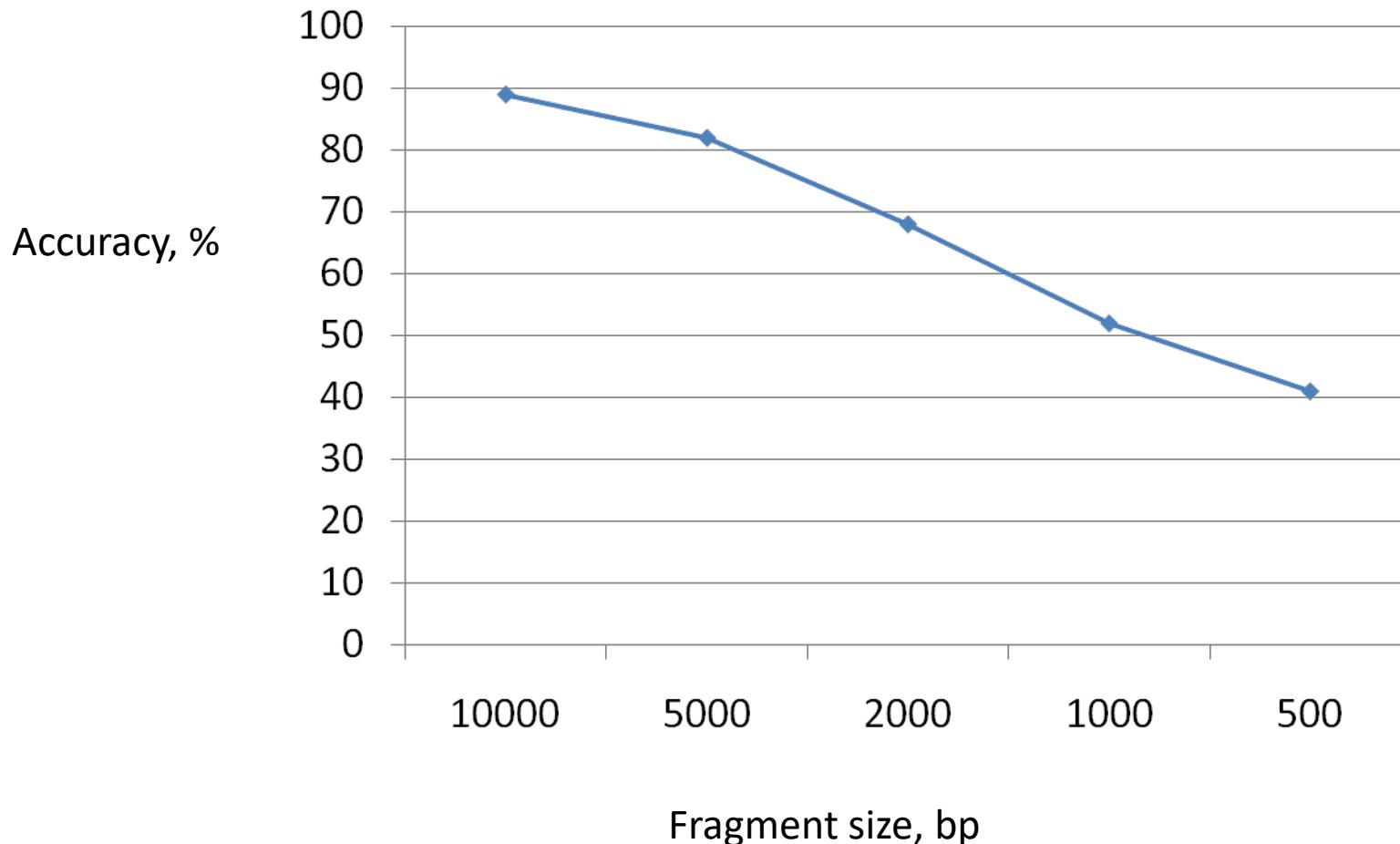
random

Explicação da imagem anterior

- Cada barra (ou bar code vertical) corresponde a 1 Mbp de um genoma de um procarioto (com exceção da última)
- cada barra pode ser entendida como a matriz de frequência desse fragmento genômico, com valores de frequência convertidos para tons de cinza
- as faixas verticais de cada barra significam k-meros de alta frequência (mais escuro) ou de baixa frequência (mais claro) ao longo desse trecho do genoma
- o fato de que existem essas faixas verticais mostra que diferentes k-meros tem diferentes frequências ao longo do genoma, e essas frequências são razoavelmente constantes ao longo do genoma

- as faixas horizontais indicam prováveis regiões de transferência horizontal, um fenômeno comum em bactérias. Essas regiões tem frequências de k-meros diferentes das frequências que caracterizam o genoma
- a última barra é uma sequência artificial, em que as bases A,T,C,G foram escolhidas aleatoriamente
 - note sua falta de estrutura
- Conclusão: podemos usar as frequências de k-meros como assinaturas genômicas
 - crie uma biblioteca com esses “códigos de barras”
 - compare as frequências de k-meros de um novo fragmento com os códigos da biblioteca; se houver “similaridade suficiente”, teremos uma identificação

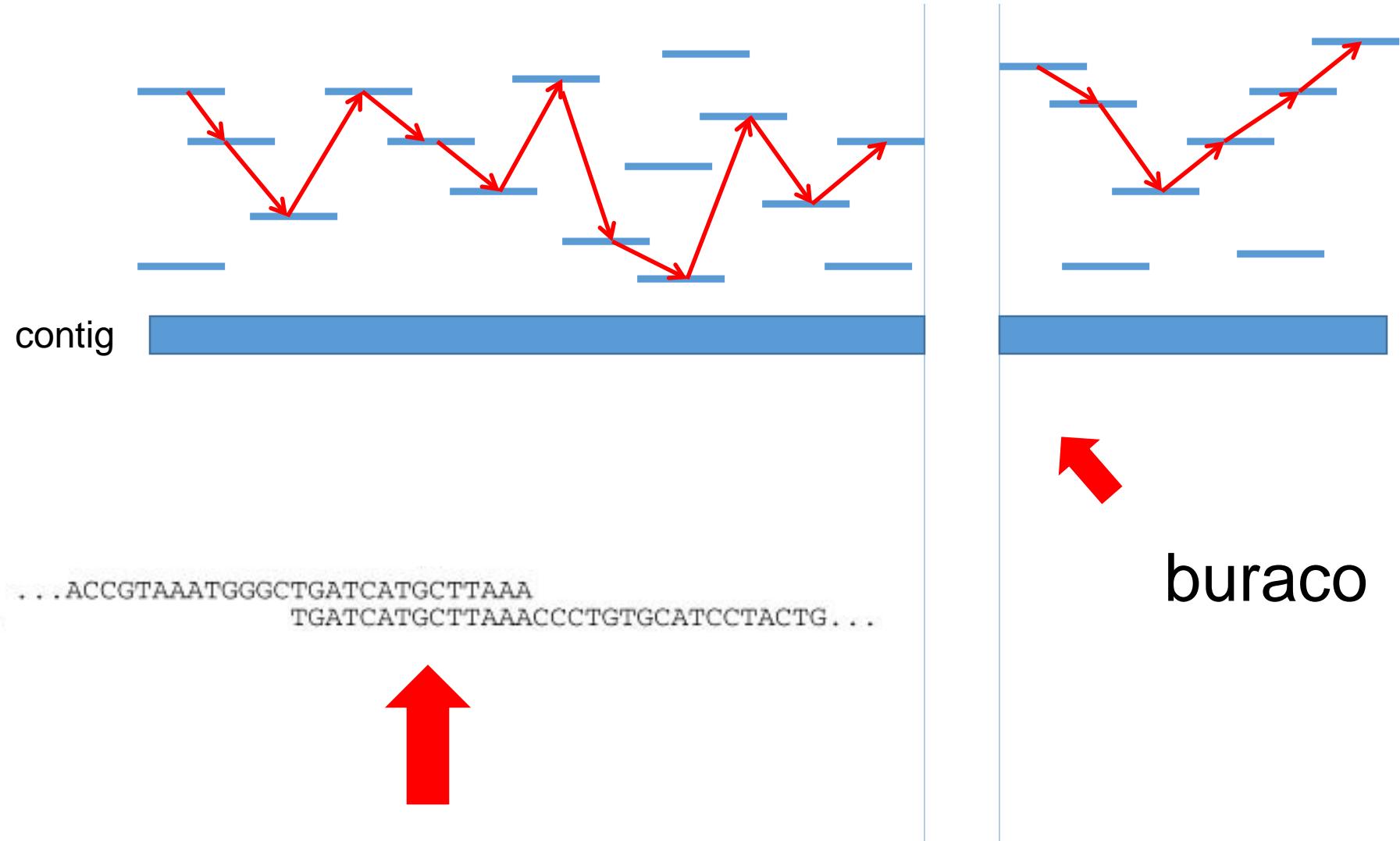
Esta técnica não funciona bem com
fragmentos **curtos**



Exercício

- Que explicação você daria para o decréscimo de acurácia com decréscimo de comprimento?

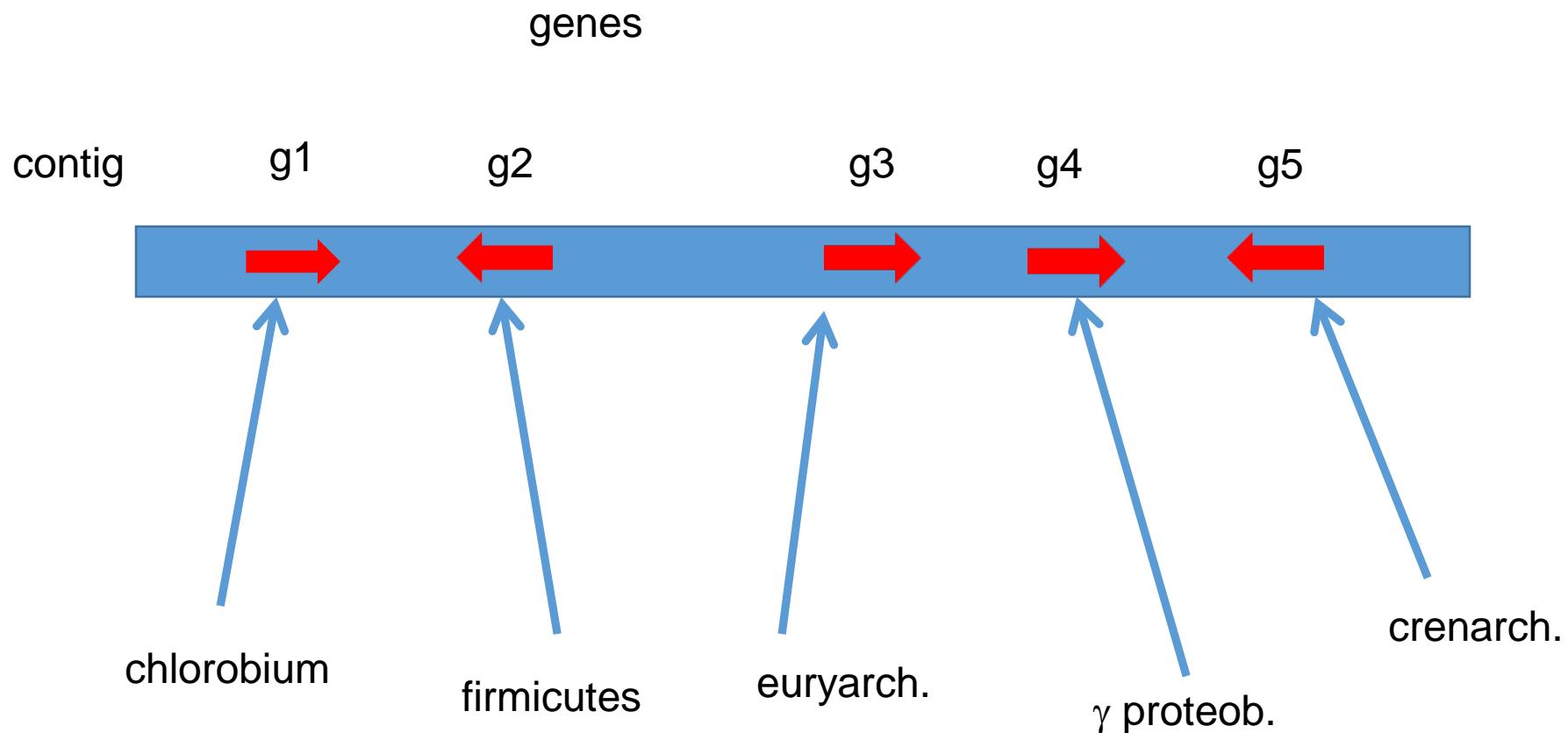
Montagem de genomas



Montagem

- Em genomas bacterianos isolados, é um processo **razoavelmente bem compreendido**
- Em metagenomas há velhas e novas dificuldades
 - Mistura de organismos
 - pode causar quimeras
 - Transferência lateral pode causar erros
 - Repetições
 - sempre um problema, especialmente se forem longas
 - Tamanho dos conjuntos de dados
 - Chegando a **bilhões** de reads

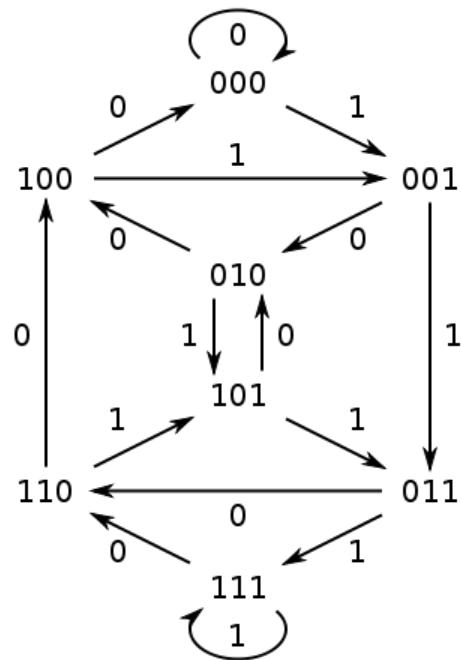
Exemplo de quimerismo: os organismos identificados são muito discrepantes entre si



Paradigmas de montagem

- OLC
 - overlap, layout, consensus
 - mais rigoroso, mas mais lento
- k-meros + grafos de de Bruijn
 - menos rigoroso, mas muito mais rápido
 - mais apropriado para metagenômica

grafos de de Bruijn



Sobreposição de k -mers

alfabeto binário

$$k = 1$$

Grafo de de Bruijn em montagem

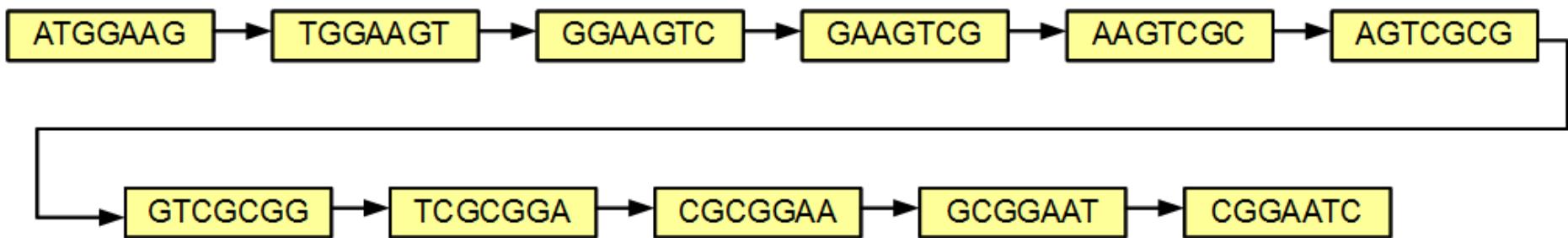
sequence

ATGGAAAGTCGCGGAATC

7mers

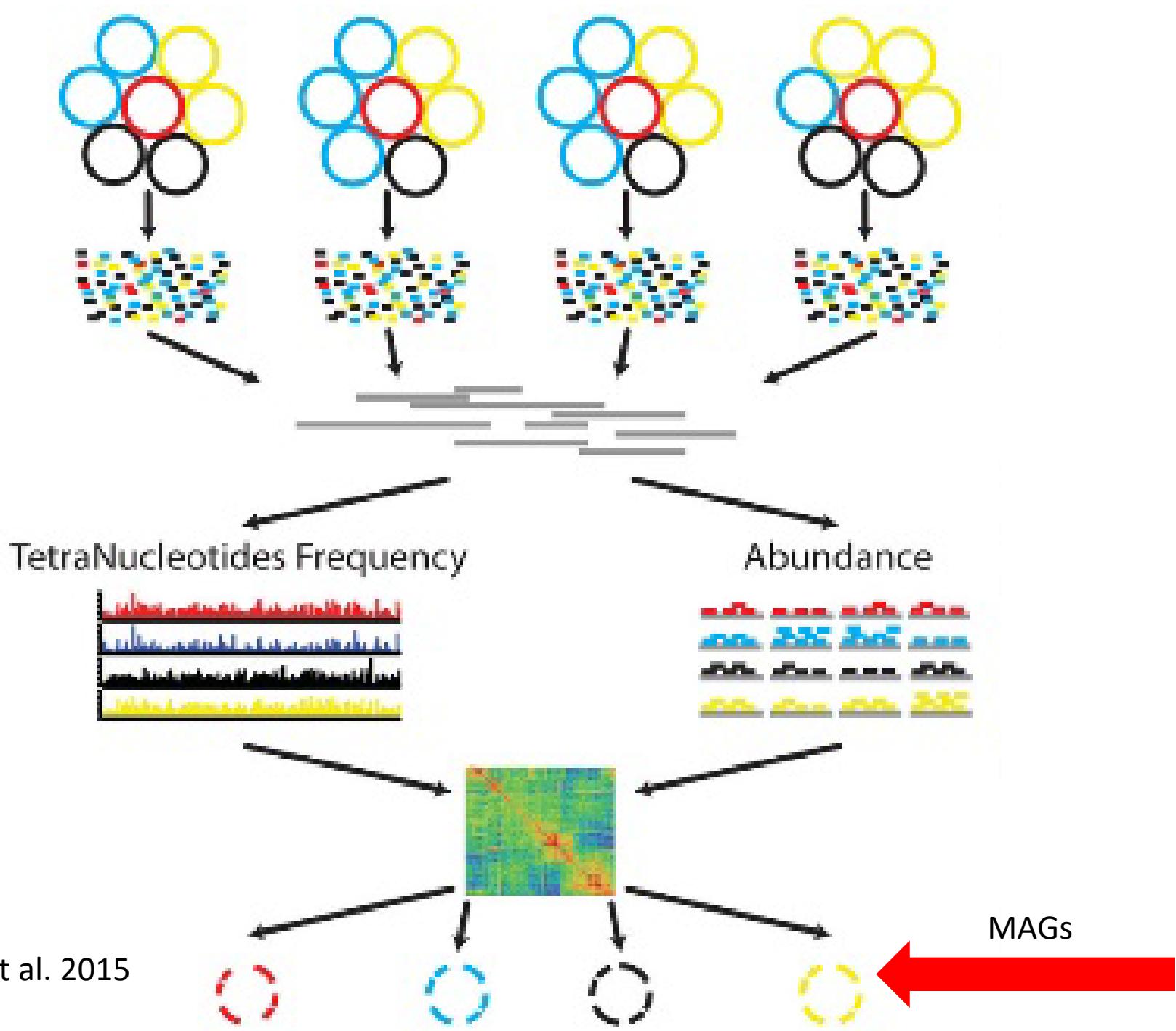
ATGGAAAG
TGGAAAGT
GGAAGTC
GAAGTCG
AAGTCGC
AGTCGCG
GTCGCGG
TCGCGGA
CGCGGAA
GCGGAAT
CGGAATC

de Bruijn graph



Metagenome-assembled genomes ou MAGs

- **Genomas** de micro-organismos obtidos a partir de sequenciamento metagenômico **shotgun** e posterior agrupamento/montagem
- Ou seja, genomas que **NÃO** são obtidos a partir de sequenciamento de **isolados**
- Tornaram-se ferramenta importante no estudo das microbiotas



explicação do diagrama anterior

- É para ler de cima para baixo
- no topo: representação dos genomas nas amostras
- sequenciamento
- montagem
- análise de contigs por tetranucleotídeos e abundância relativa
- recuperação dos genomas

MetaWRAP é um ótimo pipeline para recuperar genomas

Uritskiy et al. *Microbiome* (2018) 6:158
<https://doi.org/10.1186/s40168-018-0541-1>

Microbiome

SOFTWARE

Open Access

MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis



Gherman V. Uritskiy, Jocelyne DiRuggiero * and James Taylor *

fluxo de processamento no metaWRAP

Urtskiy et al. *Microbiome* (2018) 6:158

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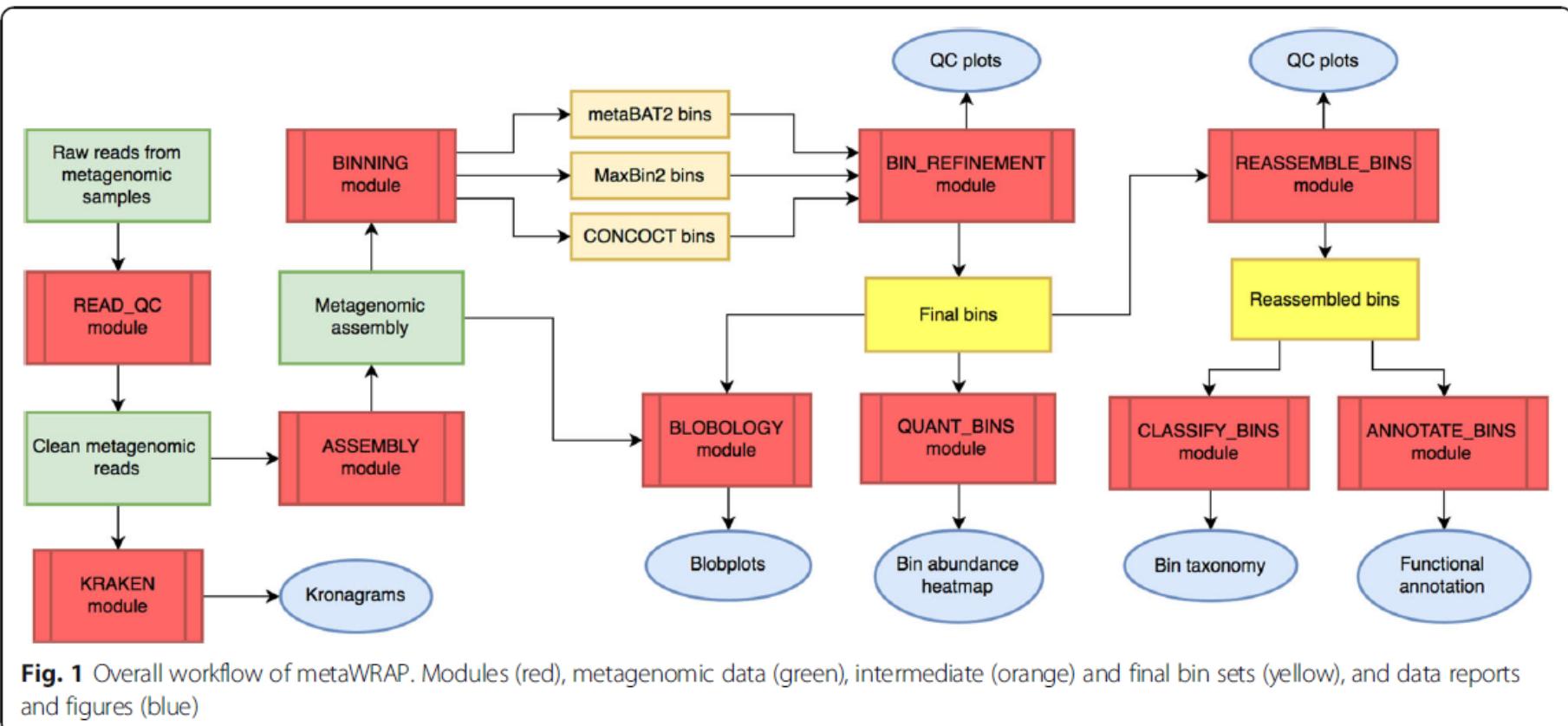


Fig. 1 Overall workflow of metaWRAP. Modules (red), metagenomic data (green), intermediate (orange) and final bin sets (yellow), and data reports and figures (blue)

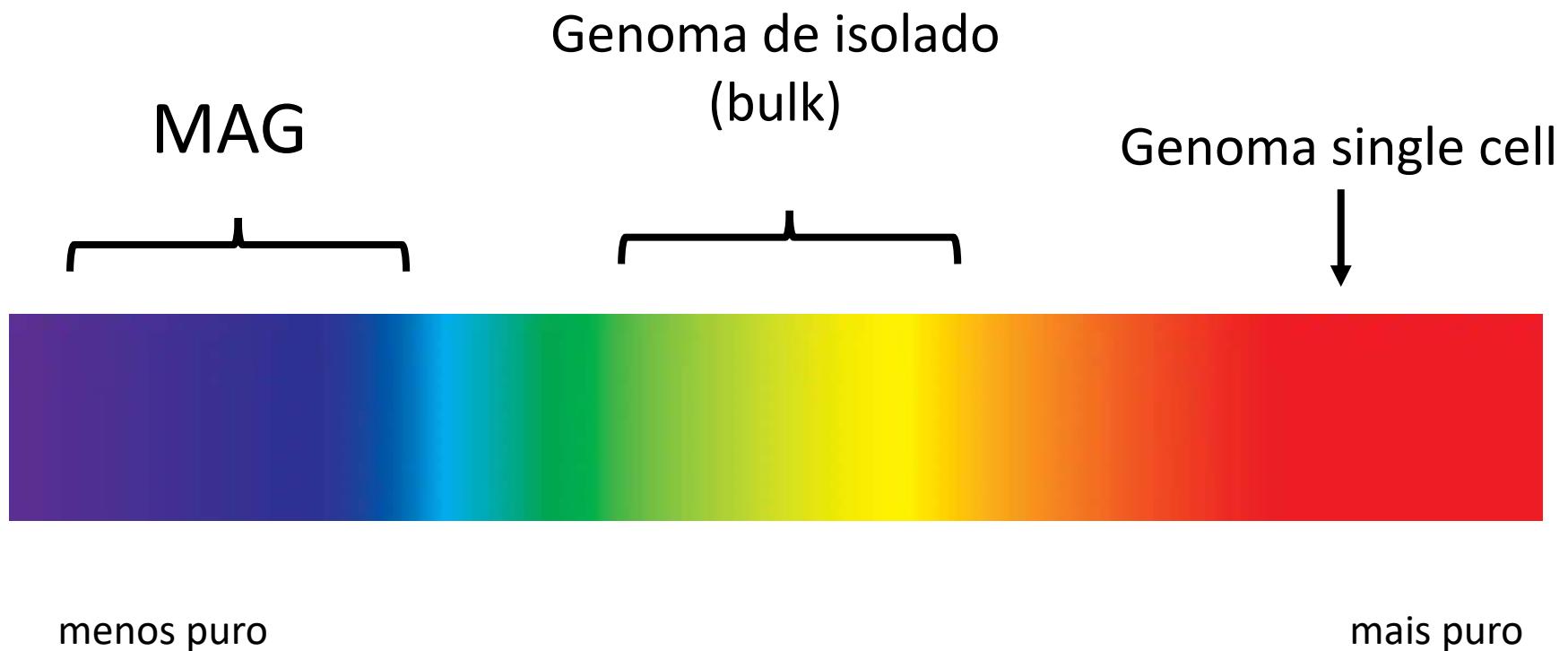
MAGs podem nos dar uma visão detalhada do ambiente de interesse

- Classificação pode chegar ao nível de **espécie**
 - Em alguns casos, de **cepas**
- abundância relativa
- **Informações sobre genes e suas funções**
- **Redes de interações**
 - Positivas e negativas
 - **Ecologia microbiana**
- Muitas oportunidades para análise computacional

MAGs são reais?

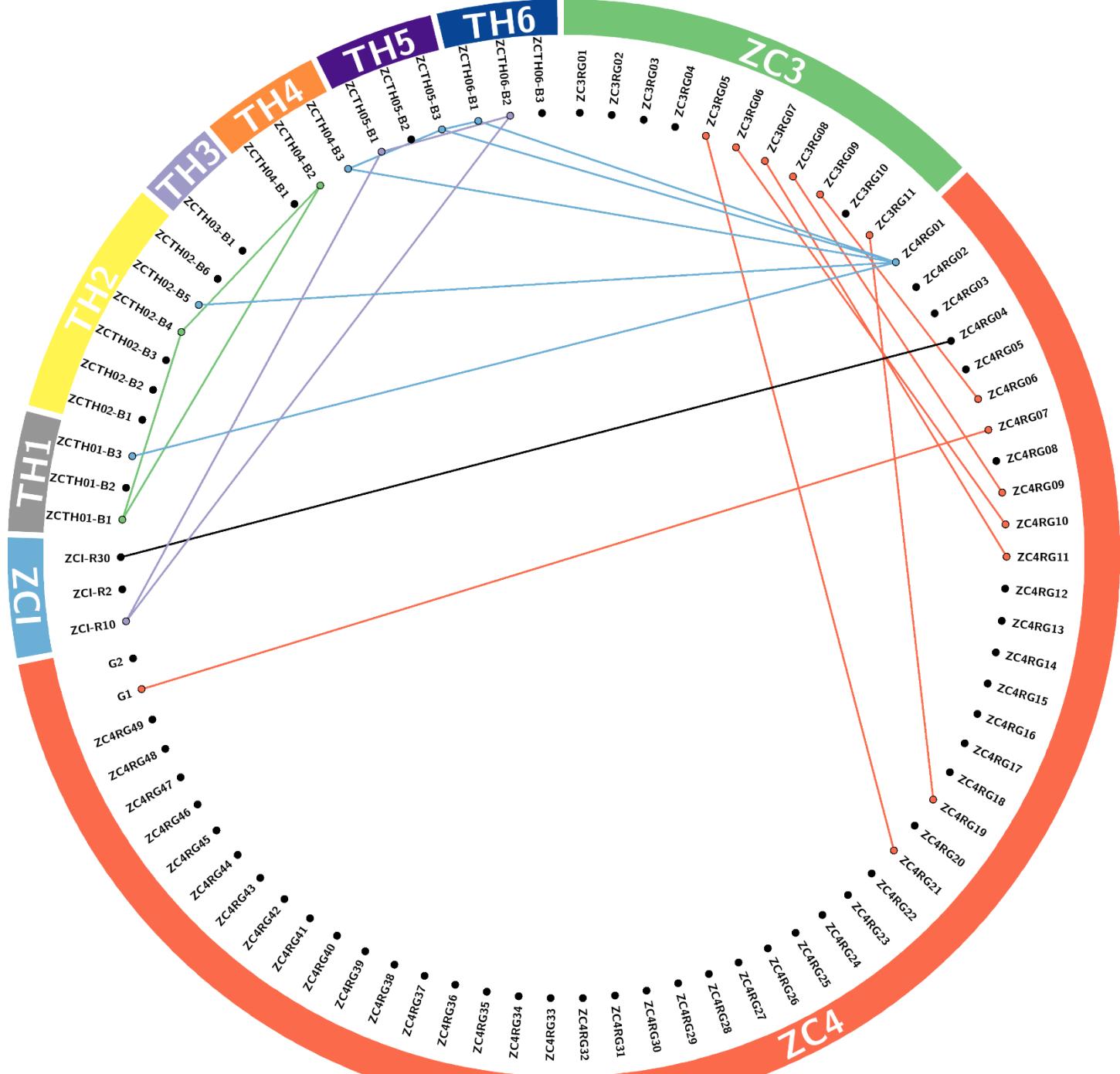
- Precisam passar por um controle de qualidade
 - Completude
 - Contaminação
 - o programa CheckM (Parks et al. 2015) faz essas estimativas
- MAGs em geral são mosaicos de cepas

O espectro de “pureza genômica”



Confirmação adicional

- Um MAG deveria poder ser recuperado de **diferentes amostras**, que sejam totalmente independentes uma da outra
- Na próxima imagem, os pontos em volta do círculo são MAGs, recuperados de diferentes amostras
- cada amostra é um arco do círculo em sua própria cor
- as linhas ligam MAGs que foram considerados “**os mesmos**” entre amostras



Classificação taxonômica de MAGs

Existe um descompasso entre taxonomia (o processo de dar rótulos válidos e universalmente aceitos a organismos) e a genômica

- genômica avança muito mais rápido do que taxonomia
- então hoje em dia existem milhares de MAGs que estão sem rótulo!
- Para lidar com esta situação, um grupo na Austrália criou um banco chamado GTDB
- Junto com o banco, eles disponibilizam uma ferramenta chamada GTDB-tk, que permite classificar um novo MAG de acordo com as informações do banco

GTDB

A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life

Donovan H Parks, Maria Chuvochina, David W Waite, Christian Rinke[✉], Adam Skarszewski,
Pierre-Alain Chaumeil & Philip Hugenholtz[✉]

Taxonomy is an organizing principle of biology and is ideally based on evolutionary relationships among organisms. Development of a robust bacterial taxonomy has been hindered by an inability to obtain most bacteria in pure culture and, to a lesser extent, by the historical use of phenotypes to guide classification. Culture-independent sequencing technologies have matured sufficiently that a comprehensive genome-based taxonomy is now possible. We used a concatenated protein phylogeny as the basis for a bacterial taxonomy that conservatively removes polyphyletic groups and normalizes taxonomic ranks on the basis of relative evolutionary divergence. Under this approach, 58% of the 94,759 genomes comprising the Genome Taxonomy Database had changes to their existing taxonomy. This result includes the description of 99 phyla, including six major monophyletic units from the subdivision of the Proteobacteria, and amalgamation of the Candidate Phyla Radiation into a single phylum. Our taxonomy should enable improved classification of uncultured bacteria and provide a sound basis for ecological and evolutionary studies.

August 2018

- há discrepâncias entre a taxonomia tradicional do NCBI e a taxonomia proposta pelo GTDB
- veja figura no próximo slide

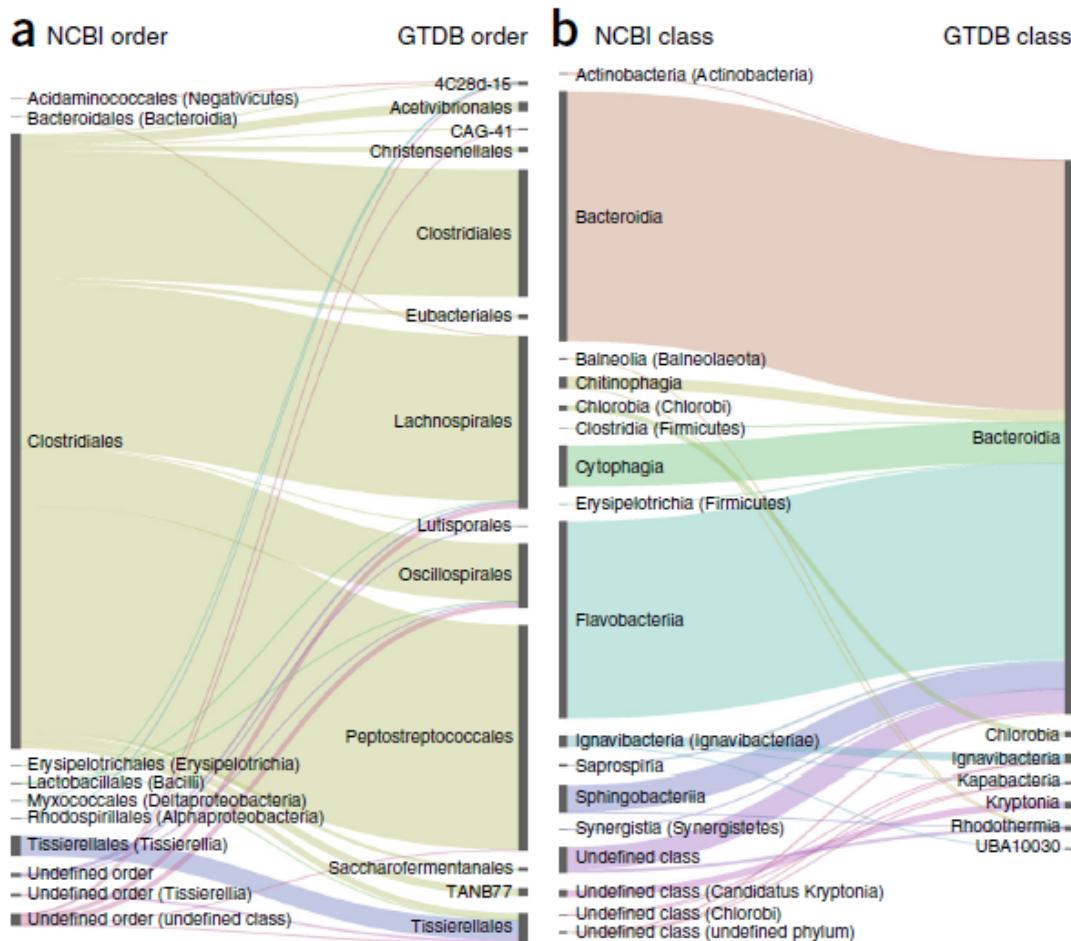


Figure 5 Comparisons of NCBI and GTDB classifications of genomes designated as Clostridia or Bacteroidetes in the GTDB taxonomy. (a) Comparison of NCBI (left) and GTDB (right) order-level classifications of the 2,368 bacterial genomes assigned to the class Clostridia in the GTDB taxonomy. Genomes classified in a class other than Clostridia by NCBI are indicated in parentheses. (b) Comparison of NCBI and GTDB class-level classifications of the 2,058 bacterial genomes assigned to the phylum Bacteroidetes in the GTDB taxonomy. Genomes classified in a phylum other than the Bacteroidetes by NCBI are indicated in parentheses.

Anotação funcional

- Pipelines para genomas completos podem ser usados em MAGs
 - IMG/M
 - RAST
 - PGAP
- Revejam aula sobre anotação de genomas

Cobertura

- Quanto cada genoma é coberto pelos reads obtidos
- Ambientes de grande riqueza: **cobertura baixa**
- Cobertura baixa cria **contigs pequenos**
 - maioria das ORFs são parciais
 - Dificulta atribuição de função
 - Potencial gerador de erros

Sumário de MAGs

- MAGs são “reais”
- Contribuem para **lançar luz** na “materia escura microbiana”
- Permitem melhor compreensão dos seus ambientes
- **Cuidado** com a representatividade dos MAGs
 - Complementar com análise de todos os reads/contigs de sua amostra, pois os MAGs representam apenas uma fração dessa massa de dados

Muitos MAGs estão sendo recuperados de amostras humanas

Resource

Cell

Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle

janeiro 2019

Edoardo Pasolli,¹ Francesco Asnicar,^{1,8} Serena Manara,^{1,8} Moreno Zolfo,^{1,8} Nicolai Karcher,¹ Federica Armanini,¹ Francesco Beghini,¹ Paolo Manghi,¹ Adrian Tett,¹ Paolo Ghensi,¹ María Carmen Collado,² Benjamin L. Rice,³ Casey DuLong,⁴ Xochitl C. Morgan,⁵ Christopher D. Golden,⁴ Christopher Quince,⁶ Curtis Huttenhower,^{4,7} and Nicola Segata^{1,9,*}

nature
biotechnology

RESOURCE

<https://doi.org/10.1038/s41587-020-0603-3>

 Check for updates

OPEN

A unified catalog of 204,938 reference genomes from the human gut microbiome

julho 2020

Alexandre Almeida^{1,2}✉, Stephen Nayfach^{3,4}, Miguel Boland¹, Francesco Strozzi^{1,5}, Martin Beracochea¹, Zhou Jason Shi^{6,7}, Katherine S. Pollard^{1,6,7,8,9,10,11}, Ekaterina Sakharova¹, Donovan H. Parks^{1,12}, Philip Hugenholtz^{1,12}, Nicola Segata^{1,13}, Nikos C. Kyriides^{1,3,4} and Robert D. Finn¹✉

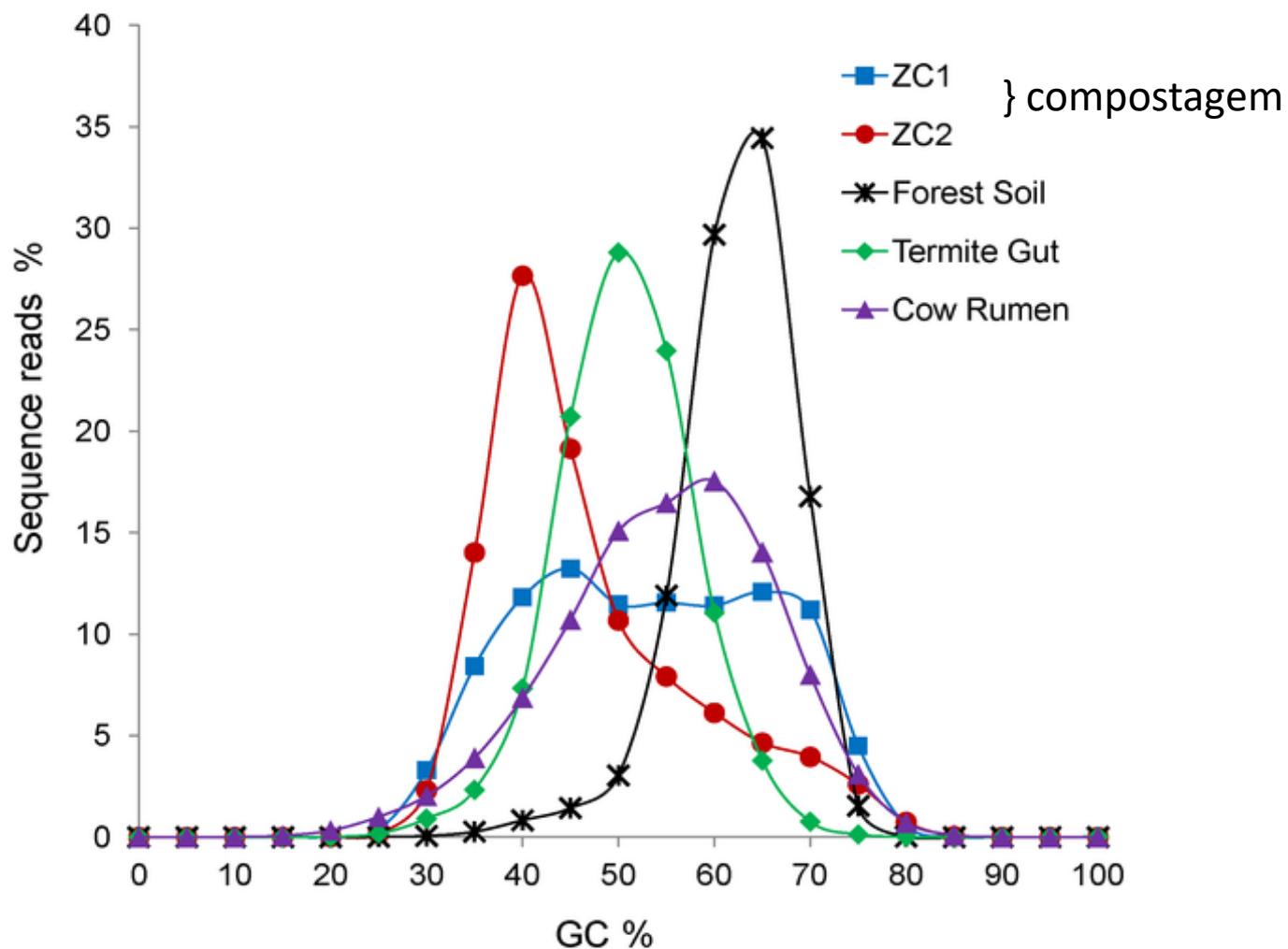
Comparação de metagenomas

- Aqui estamos falando não mais em termos de MAGs, mas em termos de coleção de reads
- Genomicamente
- Taxonomicamente
- Funcionalmente
- Recursos oferecidos pelo IMG/M

Uma comparação muito simples de se fazer é %GC

- verificamos como %GC nos reads varia para um dado metagenoma (coleção de reads que veio de um determinado ambiente – pode ser junção de mais de uma amostra)
- comparamos essa variação entre vários metagenomas
- Veja próxima figura

Figure 1. Distribution of the GC content percentage for ZC1 and ZC2 compared with selected metagenomes.



Martins LF, Antunes LP, Pascon RC, de Oliveira JCF, Digiampietri LA, et al. (2013) Metagenomic Analysis of a Tropical Composting Operation at the São Paulo Zoo Park Reveals Diversity of Biomass Degradation Functions and Organisms. PLoS ONE 8(4): e61928.
doi:10.1371/journal.pone.0061928

<http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0061928>

Outras comparações mais complexas são oferecidas pelo mecanismo de Genome clustering do IMG/M

Clustering Type:

By Function:

- COG
- Pfam
- KO

By Taxonomy:

- Class
- Family
- Genus

By Function Category:

- COG Categories
- COG Pathways
- KEGG Pathway Categories (KO)
- KEGG Pathway Categories (EC)
- KEGG Pathways (KO)
- KEGG Pathways (EC)
- Pfam Categories

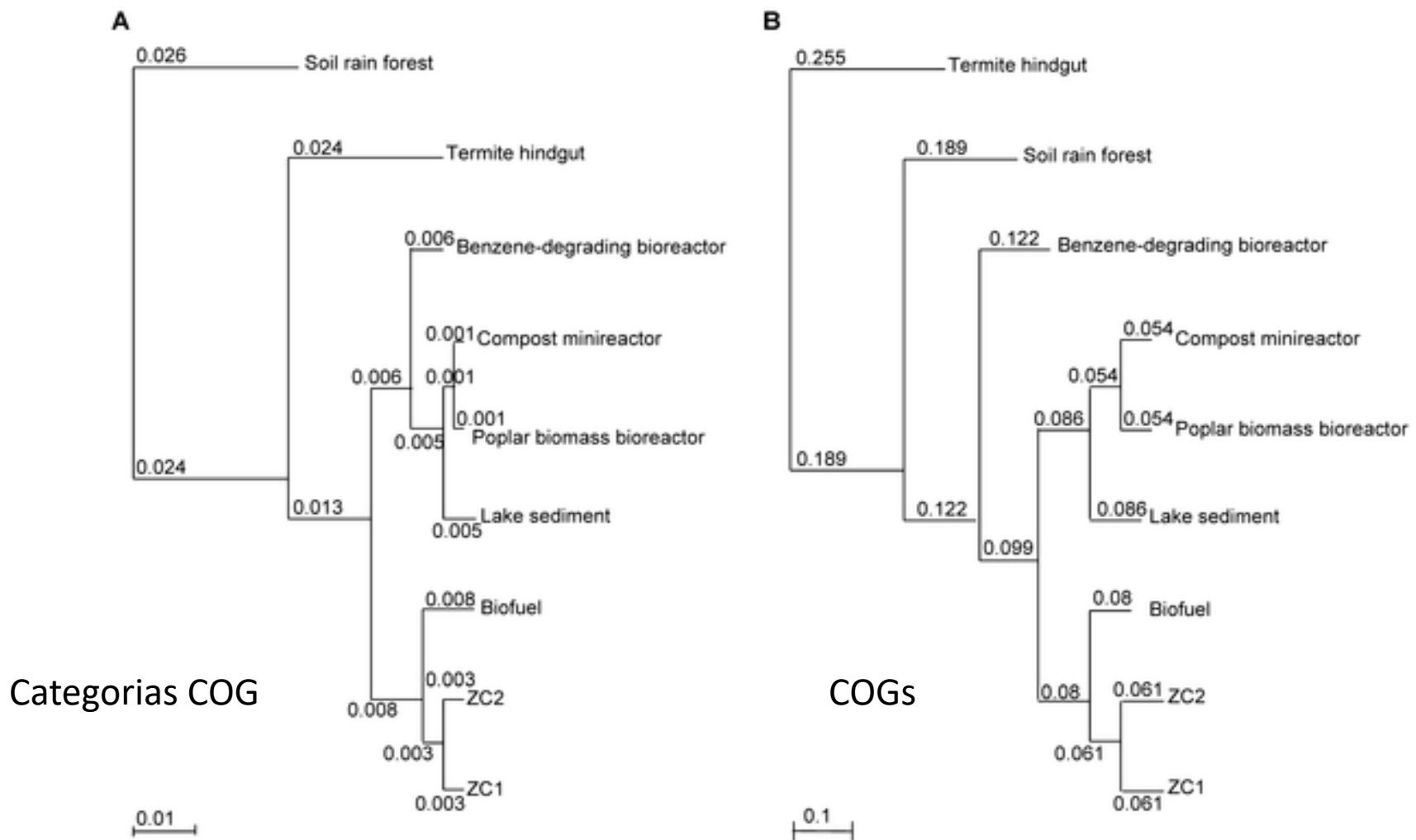
Clustering Method:

- Hierarchical Clustering
- Principal Components Analysis (PCA)
- Principal Coordinates Analysis (PCoA)
- Non-metric MultiDimensional Scaling (NMDS)
- Correlation Matrix

Go

Reset

Figure 8. Hierarchical clustering of functional gene groups of ZC1 and ZC2 and seven public metagenomes.



Martins LF, Antunes LP, Pascon RC, de Oliveira JCF, Digiampietri LA, et al. (2013) Metagenomic Analysis of a Tropical Composting Operation at the São Paulo Zoo Park Reveals Diversity of Biomass Degradation Functions and Organisms. PLoS ONE 8(4): e61928.
doi:10.1371/journal.pone.0061928

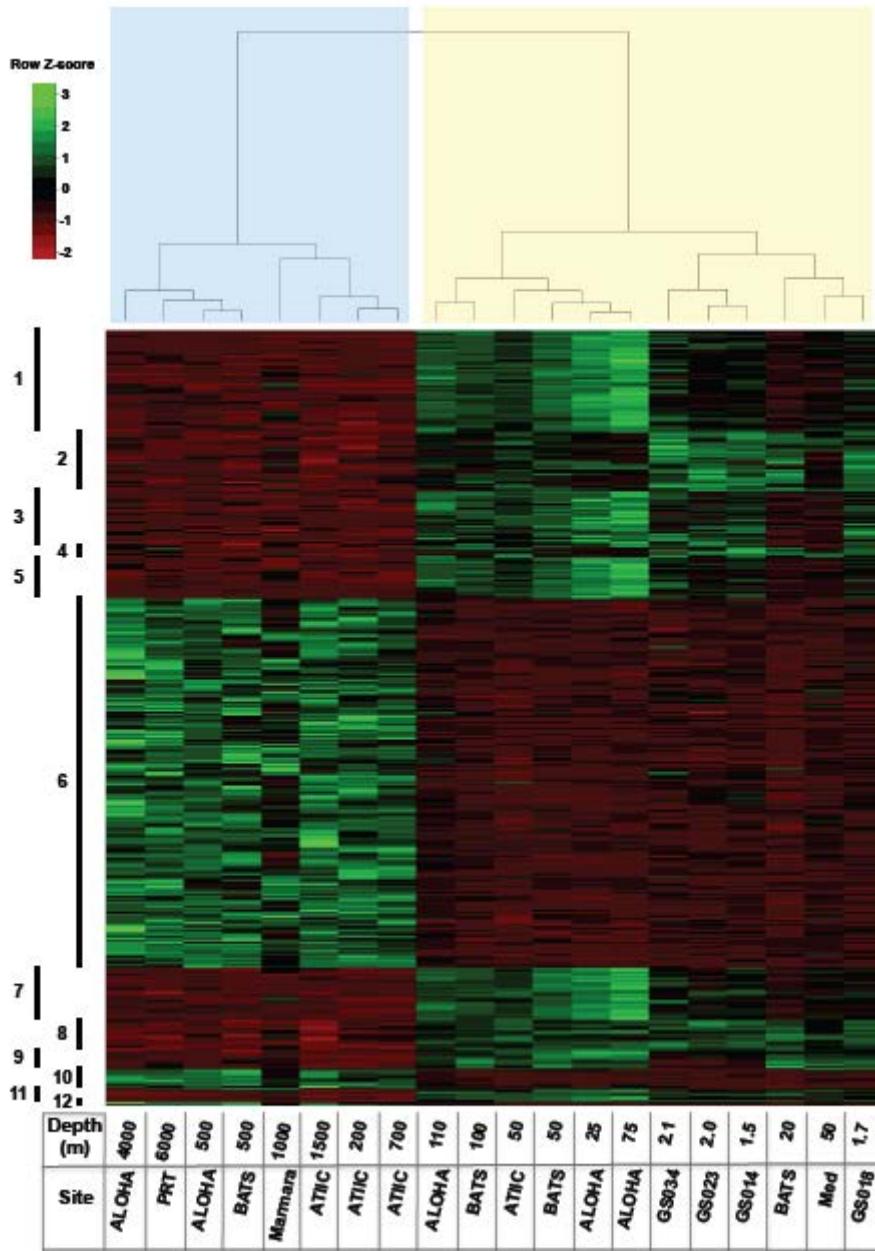
<http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0061928>

Abundância de funções

- mapeamento de reads em ORFs anotadas

Abundância relativa espacial

- é necessário o conceito de família gênica
- COG: Clusters of Orthologous Groups
- É um jeito de agrupar genes em famílias
- Se temos os genes dos metagenomas classificados por COGs, podemos computar
 - **representação diferencial** dos COGs
- Semelhante a genes diferencialmente expressos
- Ou seja, há COGs que estão mais representados (mais abundantes) em certas amostras comparadas com outras?
- Podemos representar o resultado por heat maps com clusterização hierárquica



Based on 386 COGs
shared by ATIIC, Aloha,
BATS with differential
representation

COGs

Iquique not included

Exercício

- Na imagem anterior, as linhas representam COGs e as colunas representam amostras
- a cor vermelha indica sub-representação e a cor verde indica super-representação; preto (ou escuro) significa estar próximo da media (nem sub-, nem super-)
- Quais resultados este diagrama nos mostra?

Platformas web de processamento

- Laboratórios governamentais
- Serviços padronizados de processamento

MG-RAST

metagenomics analysis server

[Browse Metagenomes](#) [About](#)[Register](#)[Contact](#)[Help](#)[Upload*](#)[News](#)

MG-RAST (the Metagenomics RAST) server is an automated analysis platform for metagenomes providing quantitative insights into microbial populations based on sequence data.

# of metagenomes	77,307
# base pairs	25.81 Tbp
# of sequences	236.94 billion
# of public metagenomes	12,527

The server primarily provides upload, quality control, automated annotation and analysis for prokaryotic metagenomic shotgun samples. MG-RAST was launched in 2007 and has over 8000 registered users and 77,307 data sets. The current server version is 3.3.3.3. We suggest users take a look at MG-RAST for the impatient.

[Updates](#)[MG-RAST 3.2.4 release notes \[October 2012\]](#)

* login required

This project has been funded in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272200900040C.

This work was supported in part by the Office of Advanced Scientific Computing Research, Office of Science, U.S. Department of Energy, under Contract DE-AC02-06CH11357.

[cite MG-RAST](#)

INTEGRATED MICROBIAL GENOMES
EXPERT REVIEW with MICROBIOME SAMPLES[IMG/M ER Home](#) [Find Genomes](#) [Find Genes](#) [Find Functions](#) [Compare Genomes](#) [Analysis Cart](#) [My IMG](#) [Companion Systems](#) [Using IMG/M ER](#)[Home](#) > Find Genomes

loaded.

Microbiome Details (Assembled Data)

[Add to Genome Cart](#)[Browse Genome](#)[BLAST Genome](#)

About Genome

- o [Overview](#)
- o [Statistics](#)
- o [Genes](#)

Overview

Proposal Name	Sao Paulo Zoo Compost
Sample Name	Sample C4
Taxon Object ID	2156126000
IMG Submission ID	2671
GOLD ID in IMG Database	Project Id: Gm0002180
External Links	
Genome type	metagenome
Sequencing Status	Draft
IMG Release	
Comment	
Sample Information	
Sample Site	Sao Paulo Zoo composting operation
Sample Collection Date	January 26, 2011
Isolation Country	Brazil
Sampling Strategy	8 days after composting started
Sample Isolation	done 8 days after composting started
Temperature Range	Thermophile
Sample Assembly Method	newbler
Sample Geographic Location	Sao Pulo Zoo
Longitude	-46.62
Latitude	-23.65

Sugestões de leitura

Analytical Tools and Databases for Metagenomics in the Next-Generation Sequencing Era

Mincheol Kim¹, Ki-Hyun Lee¹, Seok-Whan Yoon¹, Bong-Soo Kim², Jongsik Chun^{1,2}, Hana Yi^{3,4,5*}

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²Chunlab Inc., Seoul National University, Seoul 151-742, Korea, ³Department of Environmental Health, Korea University, Seoul 136-703, Korea, ⁴Department of Public Health Sciences, Graduate School, Korea University, Seoul 136-703, Korea,

⁵Korea University Guro Hospital, Korea University College of Medicine, Seoul 136-703, Korea

Metagenomics has become one of the indispensable tools in microbial ecology for the last few decades, and a new revolution in metagenomic studies is now about to begin, with the help of recent advances of sequencing techniques. The massive data production and substantial cost reduction in next-generation sequencing have led to the rapid growth of metagenomic research both quantitatively and qualitatively. It is evident that metagenomics will be a standard tool for studying the diversity and function of microbes in the near future, as fingerprinting methods did previously. As the speed of data accumulation is accelerating, bioinformatic tools and associated databases for handling those datasets have become more urgent and necessary. To facilitate the bioinformatics analysis of metagenomic data, we review some recent tools and databases that are used widely in this field and give insights into the current challenges and future of metagenomics from a bioinformatics perspective.

Keywords: computational biology, high-throughput nucleotide sequencing, metagenomics



Review

Human Microbiome Acquisition and Bioinformatic Challenges in Metagenomic Studies

Valeria D'Argenio ^{1,2,3} 

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Received: 14 December 2017; Accepted: 24 January 2018; Published: 27 January 2018

Abstract: The study of the human microbiome has become a very popular topic. Our microbial counterpart, in fact, appears to play an important role in human physiology and health maintenance. Accordingly, microbiome alterations have been reported in an increasing number of human diseases. Despite the huge amount of data produced to date, less is known on how a microbial dysbiosis effectively contributes to a specific pathology. To fill in this gap, other approaches for microbiome study, more comprehensive than 16S rRNA gene sequencing, i.e., shotgun metagenomics and metatranscriptomics, are becoming more widely used. Methods standardization and the development of specific pipelines for data analysis are required to contribute to and increase our understanding of the human microbiome relationship with health and disease status.

Keywords: human microbiome; 16S rRNA analysis; metagenomics; metatranscriptomics; data analysis; bioinformatics

Best practices for analysing microbiomes

Rob Knight^{1,4,6,12*}, Alison Vrbanac^{2,12}, Bryn C. Taylor^{2,12}, Alexander Aksenov³, Chris Callewaert^{4,5}, Justine Debelius⁴, Antonio Gonzalez⁴, Tomasz Kosciolek¹⁰, Laura-Isobel McCall³, Daniel McDonald⁴, Alexey V. Melnik³, James T. Morton^{4,6}, Jose Navas⁶, Robert A. Quinn³, Jon G. Sanders¹⁰, Austin D. Swafford¹, Luke R. Thompson¹⁰, Anupriya Tripathi⁹, Zhenjiang Z. Xu⁴, Jesse R. Zaneveld¹⁰, Qiyun Zhu¹⁰, J. Gregory Caporaso¹¹ and Pieter C. Dorrestein^{1,3,4}

Abstract | Complex microbial communities shape the dynamics of various environments, ranging from the mammalian gastrointestinal tract to the soil. Advances in DNA sequencing technologies and data analysis have provided drastic improvements in microbiome analyses, for example, in taxonomic resolution, false discovery rate control and other properties, over earlier methods. In this Review, we discuss the best practices for performing a microbiome study, including experimental design, choice of molecular analysis technology, methods for data analysis and the integration of multiple omics data sets. We focus on recent findings that suggest that operational taxonomic unit-based analyses should be replaced with new methods that are based on exact sequence variants, methods for integrating metagenomic and metabolomic data, and issues surrounding compositional data analysis, where advances have been particularly rapid. We note that although some of these approaches are new, it is important to keep sight of the classic issues that arise during experimental design and relate to research reproducibility. We describe how keeping these issues in mind allows researchers to obtain more insight from their microbiome data sets.

Exact sequence variants
For marker gene sequencing, the exact DNA sequence for each read is used instead of operational taxonomic unit clustering.

Operational taxonomic units
(OTUs). A group of closely related individuals or sequences (often 97% sequence similarity threshold).

Machine learning
The use of algorithms to learn from and make predictions about data.

Advances in DNA sequencing technologies have transformed our capacity to investigate the composition and dynamics of complex microbial communities that inhabit diverse environments, from mammalian gastrointestinal tracts to deep ocean sediments. These developments have led to vast increases in the number of microbiome studies being performed in many fields of science, from clinical research to biotechnology. With this transformation, researchers are often left holding massive amounts of data and are confronted with a bewildering array of computational tools and methods for analysing their data. Conducting a robust experiment is not trivial in microbiome research, and as with any study, experimental methods, environmental factors and analysis methods can affect results. Standards for data collection and analysis are still emerging in the field, yet many compelling results can be achieved with current practices.

and functional assignment; integration of data sets from multiple sequencing runs; and further improvement in machine learning, compositional data analysis and multi-omics analyses. However, many of the most fundamental issues that concern microbiome studies arise from statistical and experimental design issues. The most important challenge for the field is to integrate new approaches that are unique to microbiome studies, while remembering standard practices that are broadly applicable to all scientific studies.

Although it is impossible to be fully comprehensive in one article, this Review aims to provide straightforward guidelines for designing and executing a microbiome experiment and analysing the resulting data, with a particular focus on human, model organism and environmental microbiomes. We direct the reader to more specialized reviews on specific topics where these exist.

Metagenomic approaches in microbial ecology: an update on whole-genome and marker gene sequencing analyses

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Abstract

Metagenomics and marker gene approaches, coupled with high-throughput sequencing technologies, have revolutionized the field of microbial ecology. Metagenomics is a culture-independent method that allows the identification and characterization of organisms from all kinds of samples. Whole-genome shotgun sequencing analyses the total DNA of a chosen sample to determine the presence of micro-organisms from all domains of life and their genomic content. Importantly, the whole-genome shotgun sequencing approach reveals the genomic diversity present, but can also give insights into the functional potential of the micro-organisms identified. The marker gene approach is based on the sequencing of a specific gene region. It allows one to describe the microbial composition based on the taxonomic groups present in the sample. It is frequently used to analyse the biodiversity of microbial ecosystems. Despite its importance, the analysis of metagenomic sequencing and marker gene data is quite a challenge. Here we review the primary workflows and software used for both approaches and discuss the current challenges in the field.